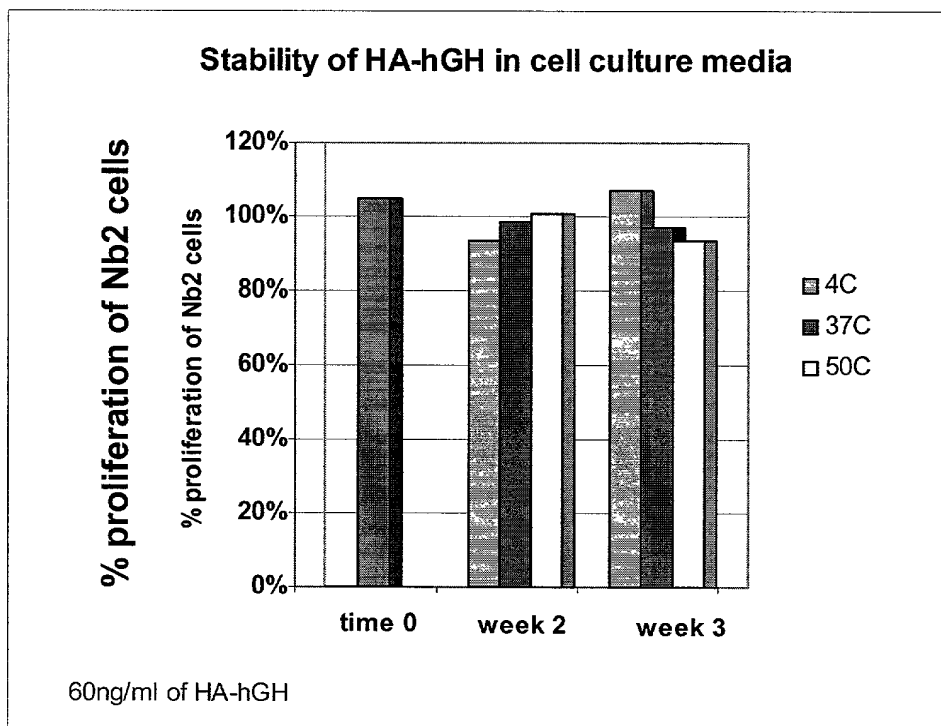
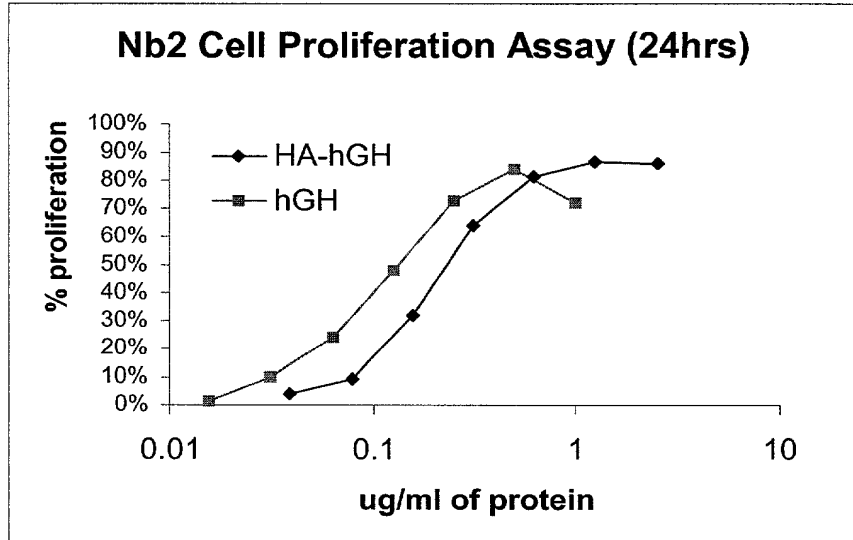
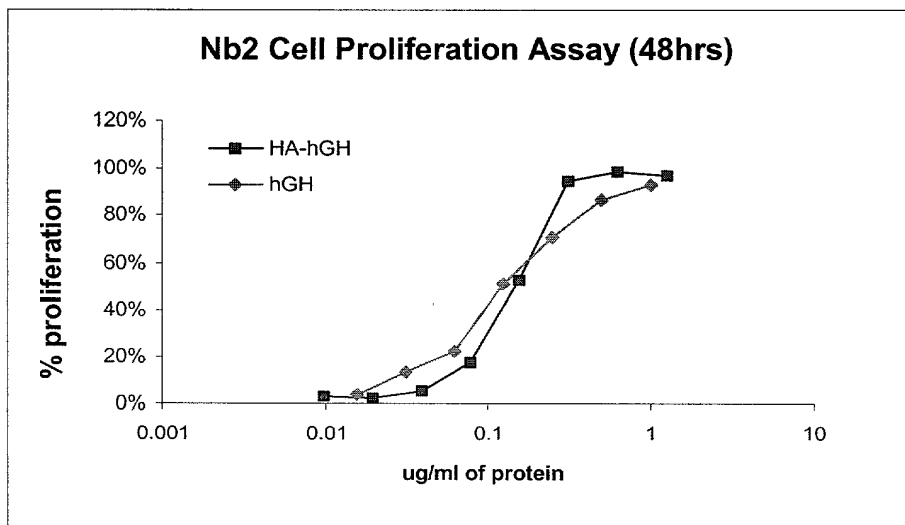


Figure 1

**Figure 2**

**Figure 3A****Figure 3B**

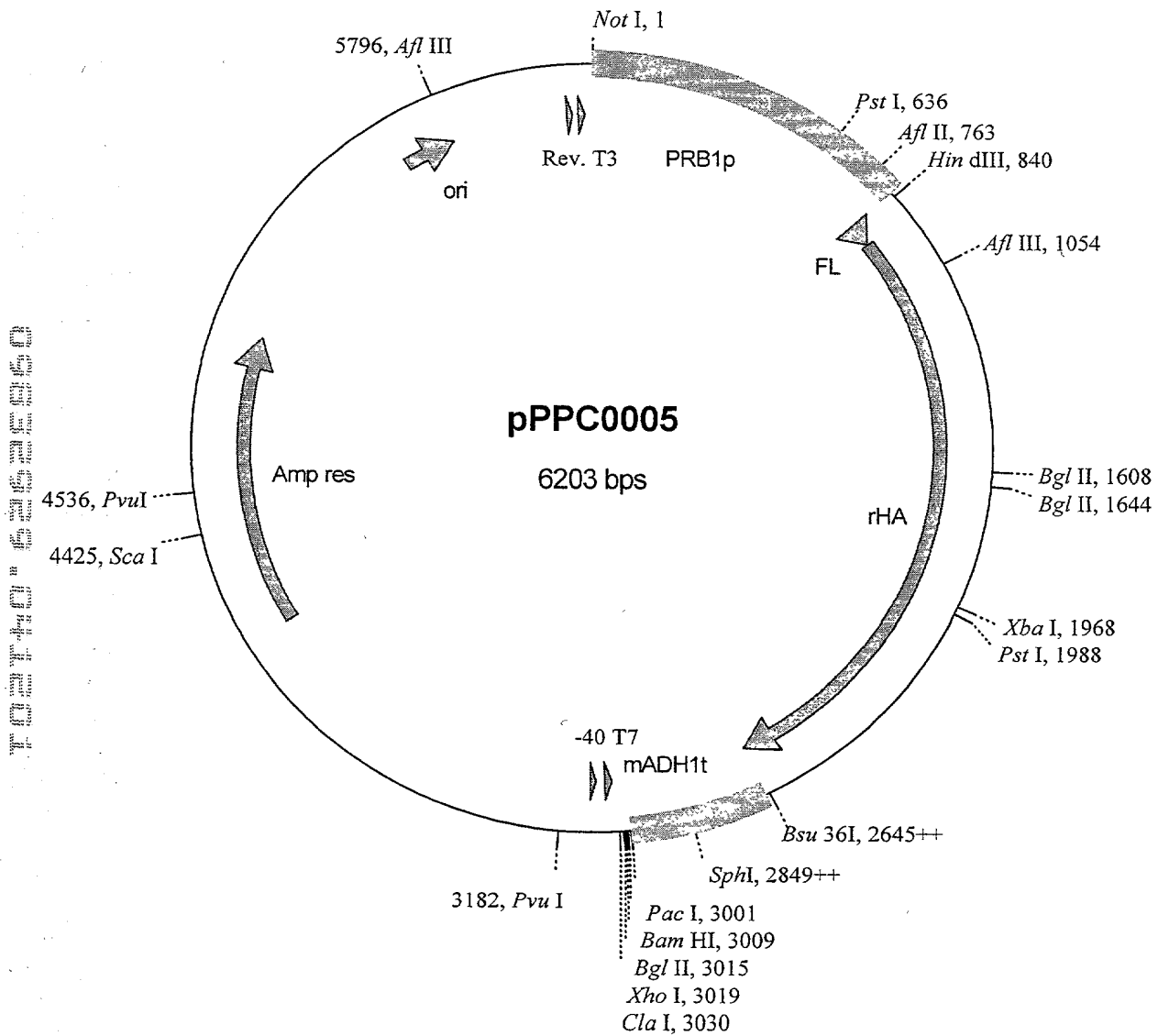


Figure 4

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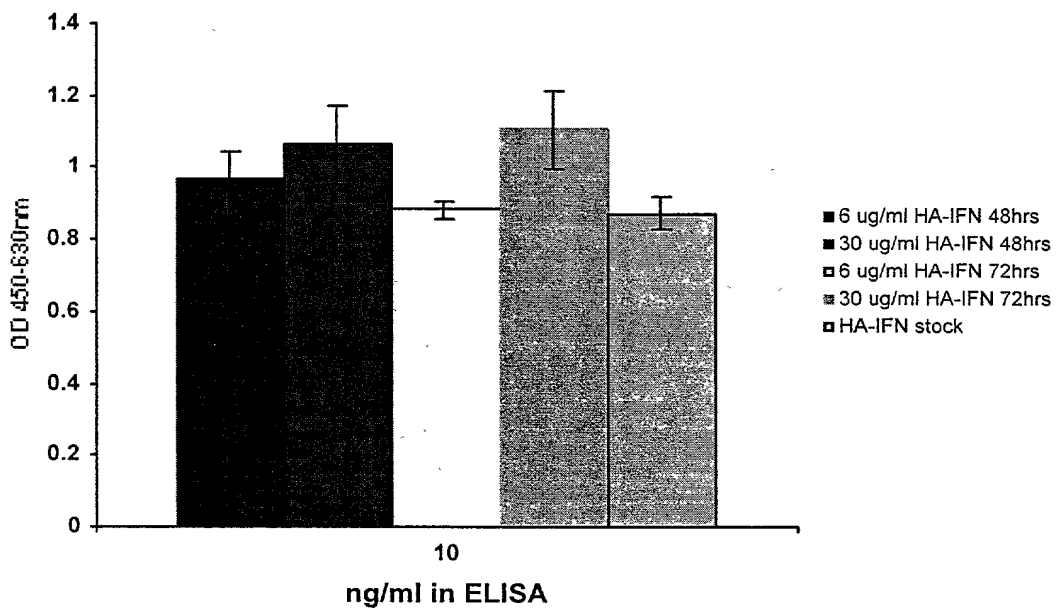
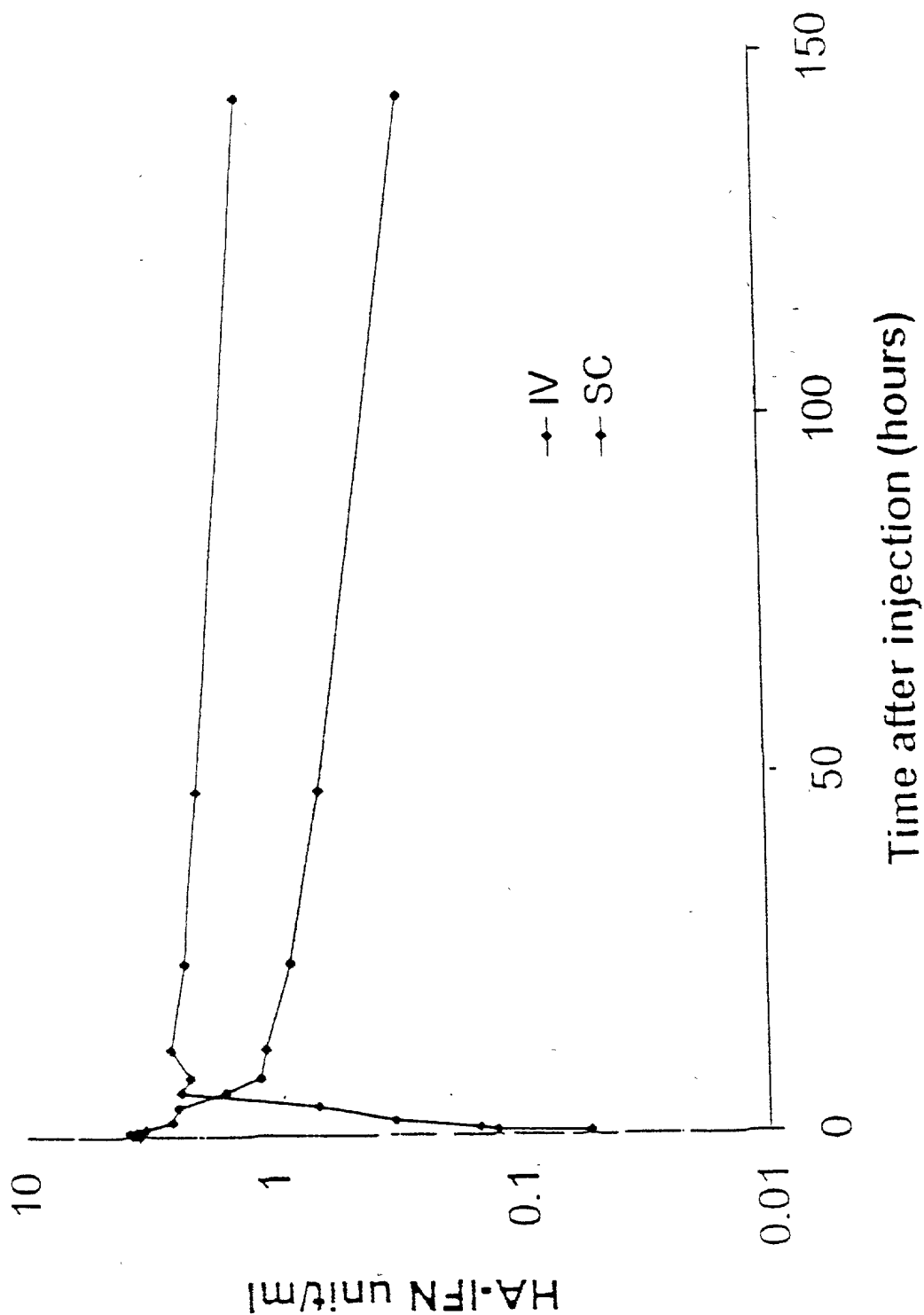
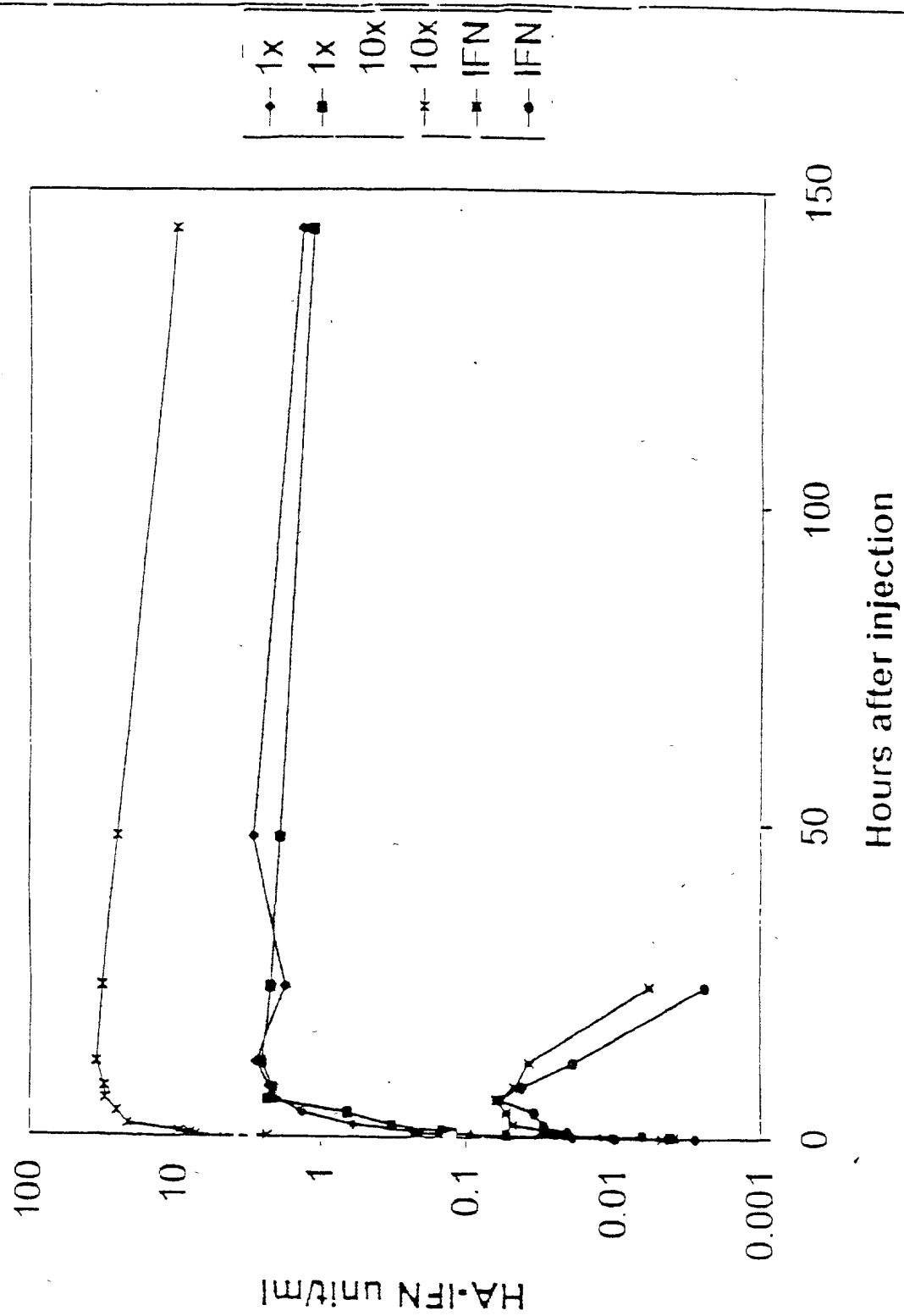


Figure 5

Figure 6





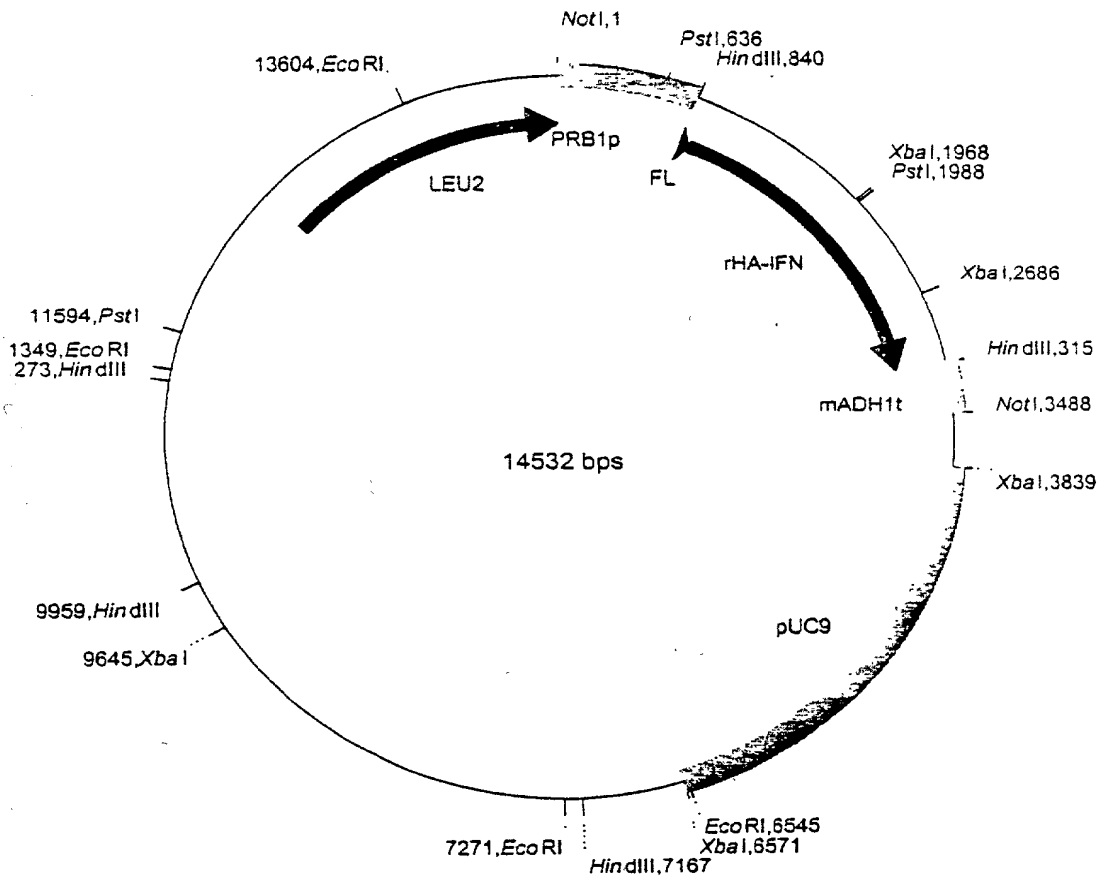


Figure 8. The HA-IFN α expression cassette in pSAC35. The expression cassette comprises
PRB1 promoter, from *S. cerevisiae*.
 Fusion leader, first 19 amino acids of the HA leader followed by the last 6 amino acids of the MF α -1 leader.
 HA-IFN α coding sequence with a double stop codon (TAATAA)
ADH1 terminator, from *S. cerevisiae*. Modified to remove all the coding sequence normally present in the *Hind* III/*Bam*HI fragment generally used.

Figure 8

Localisation of 'Loops' based on the HA Crystal Structure
which could be used for Mutation/Insertion

1	DAHKSEVAHR	FKDLGEENFK	ALVLIAFAQY	LQQCPFEDHV	KLVNEVTEFA
	HHHHH	HHH	HHH	HHHHH	HHHHHHHHHHH
	I		II		III
51	KTCVADES <u>AE</u>	NCDKSLHTLF	GDKLCTVATL	RETYGEMADC	CAKOE <u>PERNE</u>
	HHHHH	HHHHH	HHHHH	HHHH	H HHHH
101	CFLQHKDDNP	NLPRLVRPEV	DVMCTAFHDN	EETFLKKYLY	EIARRHPYFY
	HHHH	H	HHHHHHHHH	HHHHHHHHHH	HHHHH
	IV				
151	APELLFFAKR	YKAAFT <u>ECO</u>	AADKAACLLP	KLDEL RDEGK	ASSAKQRLKC
	HHHHHHHHHHH	HHHHHHHHH	HHHHH	HHHEHHHHHHH	HHHHHHHHHHH
	V				
201	ASLQKFGERA	FKAWAVARLS	QRFPKAEFAE	VSKLVTDLT <u>K</u>	VHTECC <u>HGDL</u>
	HHHHH	HH	HHHHHHHHHHH	HH	HHH HHHHHHHHHHH HHHHHH HH
	VI		VII		
251	LECADDRADL	AKYIC <u>ENODS</u>	ISSKLKECCE	KPLLEKSHCI	AEVENDEMPA
	HHHHHHHHHHH	HHHHH	HHHHH	HHHHHHHH	H
301	DLPSLAADFV	ESKDVCCKNYA	EAKDVFLGMF	LYEYARRHPD	YSVVLLRLA
	HHHH	HHHHHH	HHHHHHH	HHHHHH	HHHHHHHHH
	VIII				
351	KTYETTLEKC	CAAAD <u>PHECY</u>	AKVFDEFKPL	VEEPQNLIKQ	NCELFEOQLGE
	HHHHHHHHHHH	HH	H	HHHHH	HHHHHHHHHHH HHHHHHH
	IX				
401	YKFQNALLVR	YTKKVPQVST	PTLVEVSRNL	GKVGSKCCKH	PEAKRMPCAE
	HHHHHHHHHHH	HHHH	H	HHHHHHHHHHH	HHH HHHHHHHH
	X		XI		
451	DYLSVVLNQL	CVLHEKTPVS	DRVTKCCTES	LVNRRPPCFSA	LEVDETYVPK
	HHHHHHHHHHH	HHHHH	HHHHHHHHH	HHHHHHHHH	
501	EFNAETFTFH	ADICTLSEKE	RQIKKQTALV	ELVKHKPKAT	KEQLKAVMDD
		HHH	HHH	HHHHHMEHHH	HHH HHHHHHHH
	XII				
551	FAAFVEKCKK	ADDKETCFAE	EGKKLVAA <u>SQ</u>	AALGL	
	HHHHHHHHH	HHHH	HHHHHHHHHHH	HH	

Loop	Loop
I Val54-Asn61	VII Glu280-His288
II Thr76-Asp89	VIII Ala362-Glu368
III Ala92-Glu100	IX Lys439-Pro447
IV Gln170-Ala176	X Val462-Lys475
V His247-Glu252	XI Thr478-Pro486
VI Glu266-Glu277	XII Lys560-Thr566

Figure 9

Examples of Modifications to Loop IV

a. Randomisation of Loop IV.

IV
 151 APELLFFAKR YKAAFTECCQ AADKAACLLP KLDEL RDEGK ASSAKQRLKC
 HHHHHHHHHH HHHHHHHHHH HHHHH HHHHHHHHHHH HHHHHHHHHHH

IV
 151 APELLFFAKR YKAAFTECCX XXXXXXCLLP KLDEL RDEGK ASSAKQRLKC
 HHHHHHHHHH HHHHHHHHHH HHHHH HHHHHHHHHHH HHHHHHHHHHH

X represents the mutation of the natural amino acid to any other amino acid. One, more or all of the amino acids can be changed in this manner. This figure indicates all the residues have been changed.

b. Insertion (or replacement) of Randomised sequence into Loop IV.

$(X)_n$



IV

151 APELLFFAKR YKAAFTECCQ AADKAACLLP KLDEL RDEGK ASSAKQRLKC
 HHHHHHHHHH HHHHHHHHHH HHHHH HHHHHHHHHHH HHHHHHHHHHH

The insertion can be at any point on the loop and the length a length where n would typically be 6, 8, 12, 20 or 25.

Figure 10

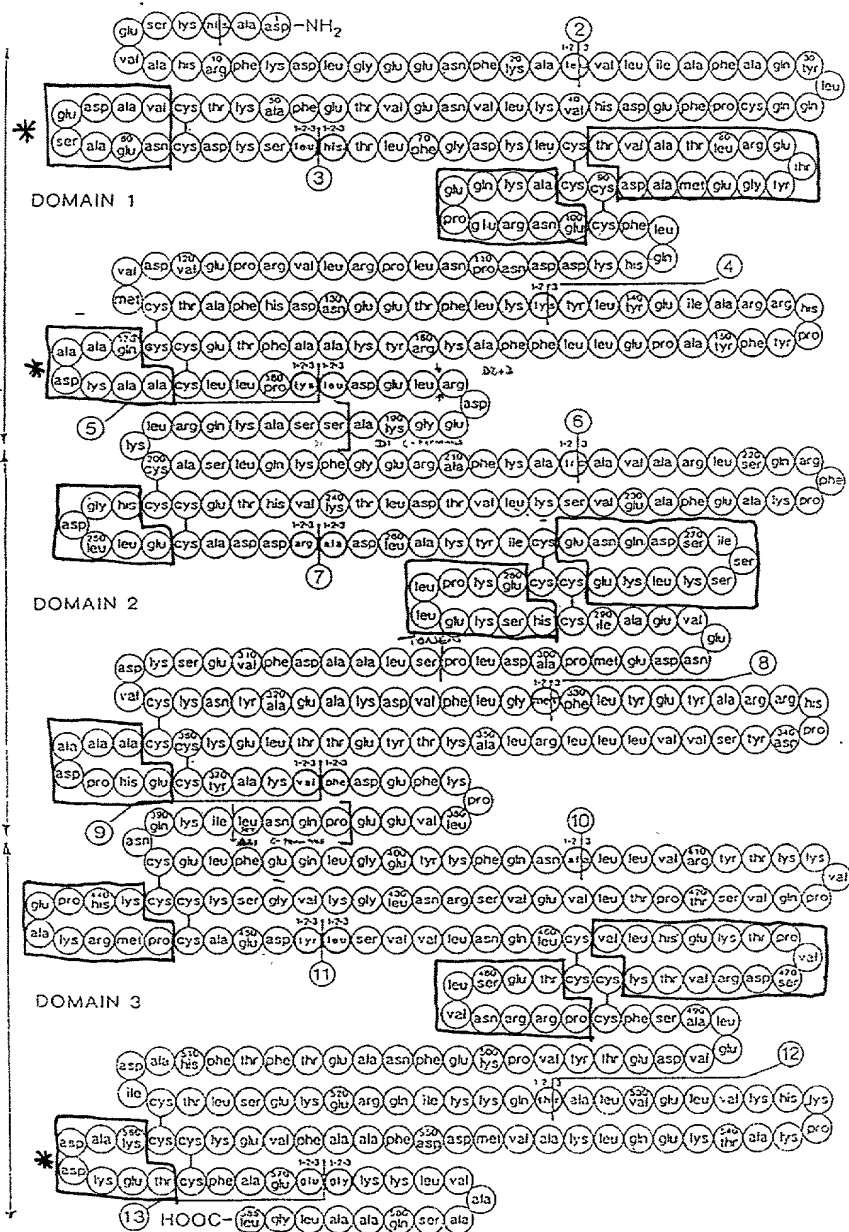
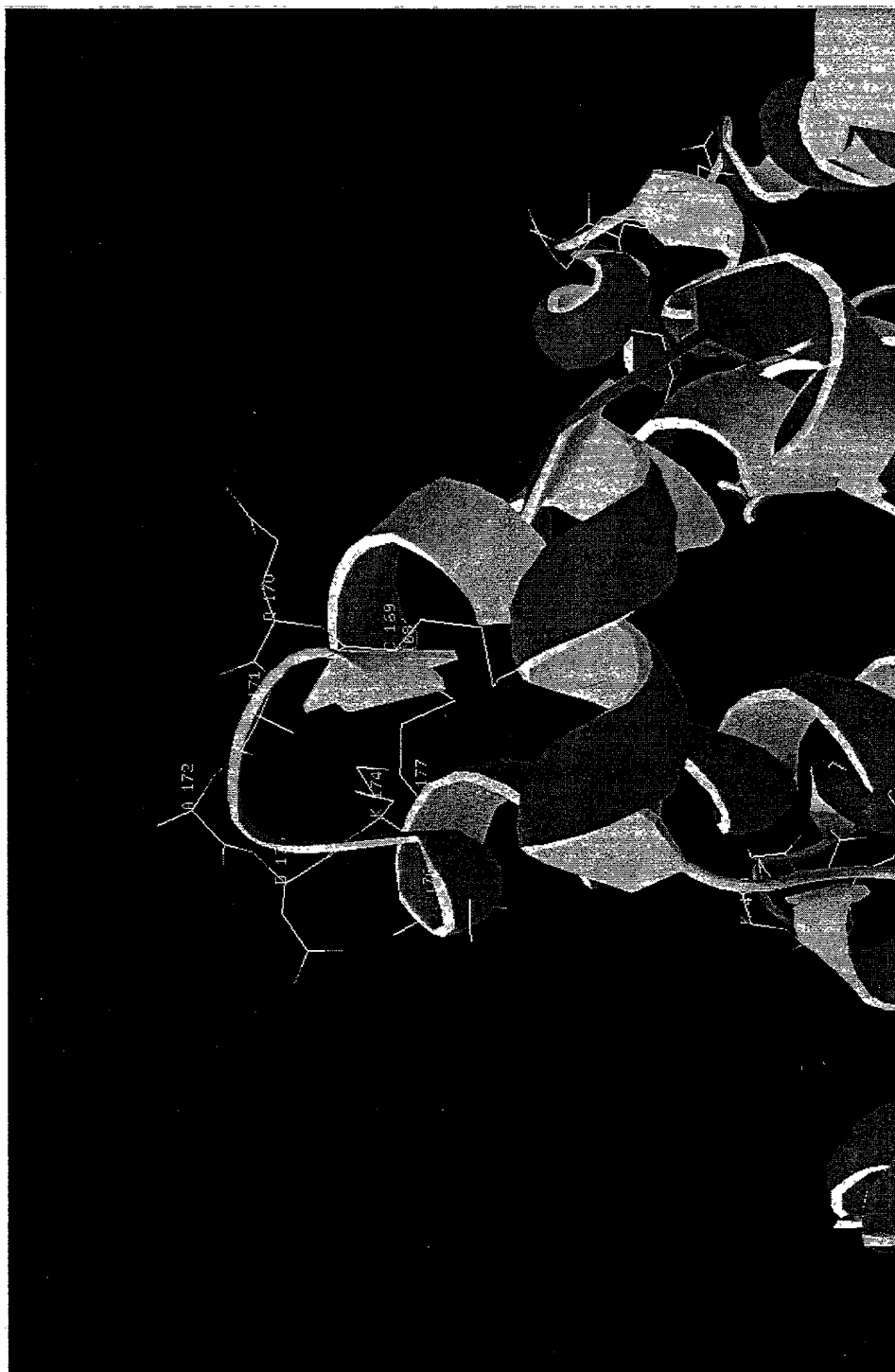


Figure 11



Disulfide bonds shown in yellow

Figure 12: Loop IV Gln170-Ala176



Figure 13: Tertiary Structure of HA

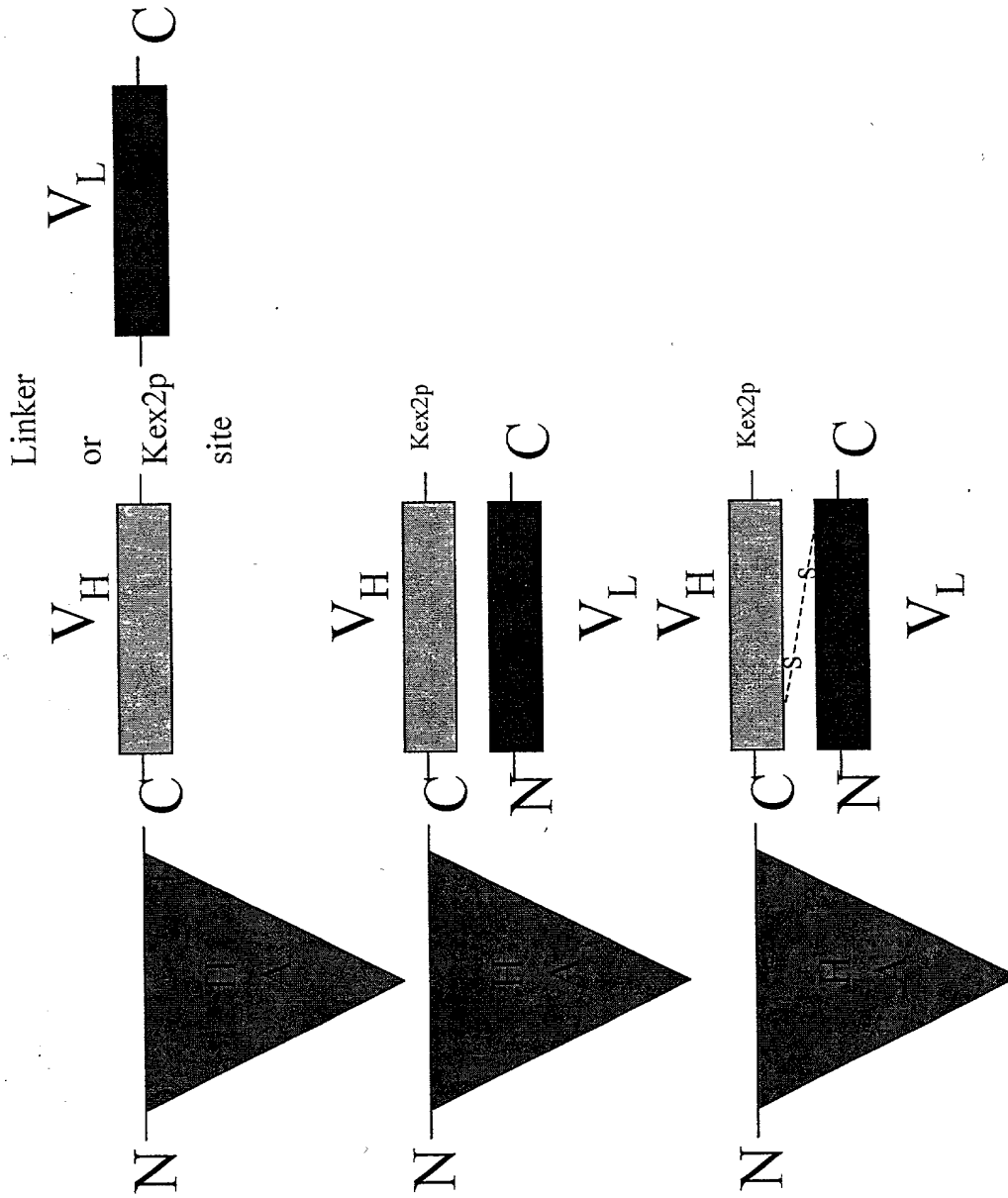


Figure 14: Schematic Diagram of Possible ScFv Fusions
 (Example is of a C-terminal fusion to HA)

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1 GAT GCA CAC AAG AGT GAG GTT GCT CAT CGG TTT AAA GAT TTG GGA GAA AAT TTC AAA 60
1 D A H K S E V A H R F K D L G E E N F K 20

61 GCC TTG GTG TTG ATT GCC TTT GCT CAG TAT CTT CAG CAG TGT CCA TTT GAA GAT CAT GTA 120
21 A L V L I A F A Q Y L Q Q C P F E D H V 40

121 AAA TTA GTG AAT GAA GTA ACT GAA TTT GCA AAA ACA TGT GTT GCT GAT GAG TCA GCT GAA 180
41 K L V N E V T E F A K T C V A D E S A E 60

181 AAT TGT GAC AAA TCA CTT CAT ACC CTT TTT GGA GAC AAA TTA TGC ACA GTT GCA ACT CTT 240
61 N C D K S L H T L F G D K L C T V A T L 80

241 CGT GAA ACC TAT GGT GAA ATG GCT GAC TGC TGT GCA AAA CAA GAA CCT GAG AGA AAT GAA 300
81 R E T Y G E M A D C C A K Q E P E R N E 100

301 TGC TTC TTG CAA CAC AAA GAT GAC AAC CCA AAC CTC CCC CGA TTG GTG AGA CCA GAG GTT 360
101 C F L Q H K D D N P N L P R L V R P E V 120

361 GAT GTG ATG TGC ACT GCT TTT CAT GAC AAT GAA GAG ACA TTT TTG AAA AAA TAC TTA TAT 420
121 D V M C T A F H D N E E T F L K K Y L Y 140

421 GAA ATT GCC AGA AGA CAT CCT TAC TTT TAT GCC CCG GAA CTC CTT TTC TTT GCT AAA AGG 480
141 E I A R R R H P Y F Y A P E L L F F A K R 160

```

Figure 15A

481 TAT AAA GCT GCT TTT ACA GAA TGT TGC CAA GCT GCT GAT AAA GCT GCC TGC CTG TTG CCA 540
 161 Y K A A F T E C C Q A A D K A A C L L P 180

 541 AAG CTC GAT GAA CTT CGG GAT GAA GGG AAG GCT TCG TCT GCC AAA CAG AGA CTC AAA TGT 600
 181 K L D E L R D E G K A S S A K Q R L K C 200

 601 GCC AGT CTC CAA AAA TTT GGA GAA AGA GCT TTC AAA GCA TGG GCA GTG GCT CGC CTG AGC 660
 201 A S L Q K K F G E R A F K A W A V A R L S 220

 661 CAG AGA TTT CCC AAA GCT GAG TTT GCA GAA GTT TCC AAG TTA GTG ACA GAT CTT ACC AAA 720
 221 Q R F P K A E F A E V S K L V T D L T K 240

 721 GTC CAC ACG GAA TGC TGC CAT GGA GAT CTG CTT GAA TGT GCT GAT GAC AGG GCG GAC CTT 780
 241 V H T E C C H G D L L E C A D D R A D L 260

 781 GCC AAG TAT ATC TGT GAA AAT CAG GAT TCG ATC TCC AGT AAA CTG AAG GAA TGC TGT GAA 840
 261 A K Y I C E N Q D S I S S K L K E C C E 280

 841 AAA CCT CTG TTG GAA AAA TCC CAC TGC ATT GCC GAA GTG GAA AAT GAT GAG ATG CCT GCT 900
 281 K P L L E K S H C I A E V E N D E M P A 300

 901 GAC TTG CCT TCA TTA GCT GCT GAT TTT GTT GAA AGT AAG GAT GTT TGC AAA AAC TAT GCT 960
 301 D L P S L A A D F V E S K D V C K N Y A 320

Figure 15B

961 GAG GCA AAG GAT GTC TTC CTG GGC ATG TTT TTG TAT GAA TAT GCA AGA AGG CAT CCT GAT 1020
 321 E A K A G D V F L G M F L Y E Y A R R H P D 340

 1021 TAC TCT GTC GTG CTG CTG CTG AGA CTT GCC AAG ACA TAT GAA ACC ACT CTA GAG AAG TGC 1080
 341 Y S V V L L L L R L A K T Y E T L E K C 360

 1081 TGT GCC GCT GCA GAT CCT CAT GAA TGC TAT GCC AAA GTG TTC GAT GAA TTT AAA CCT CTT 1140
 361 C A A A D P H E C Y A K V F D E F K P L 380

 1141 GTG GAA GAG CCT CAG AAT TTA ATC AAA CAA AAC TGT GAG CTT TTT GAG CAG CTT GGA GAG 1200
 381 V E E P Q N L I K Q N C E L F E Q L G E 400

 1201 TAC AAA TTC CAG AAT GCG CTA TTA GTT CGT TAC ACC AAG AAA GTA CCC CAA GTG TCA ACT 1260
 401 Y K F Q N A L L L V R Y T K K V P Q V S T 420

 1261 CCA ACT CTT GTA GAG GTC TCA AGA AAC CTA GGA AAA GTG GGC AGC AAA TGT TGT AAA CAT 1320
 421 P T L V E V S R N L G K V G S K C C K H 440

 1321 CCT GAA GCA AAA AGA ATG CCC TGT GCA GAA GAC TAT CTA TCC GTG GTC CTG AAC CAG TTA 1380
 441 P E A K R M P C A E D Y L S V V L N Q L 460

 1381 TGT GTG TTG CAT GAG AAA ACG CCA GTA AGT GAC AGA GTC ACA AAA TGC TGC ACA GAG TCC 1440
 461 C V L H E K T P V S D R V T K C C T E S 480

Figure 15C

1441 TTG GTG AAC AGG CGA CCA TGC TTT TCA GCT CTG GAA GTC GAT GAA ACA TAC GTT CCC AAA 1500
 481 L V N R R P C F S A L E V D E T Y V P K 500

 1501 GAG TTT AAT GCT GAA ACA TTC ACC TTC CAT GCA GAT ATA TGC ACA CTT TCT GAG AAG GAG 1560
 501 E F N A E T F T F H A D I C T L S E K E 520

 1561 ACA CAA ATC AAG AAA CAA ACT GCA CTT GTT GAG CTT GTG AAA CAC AAG CCC AAG GCA ACA 1620
 521 R Q I K K Q T A L V E L V K H K P K A T 540

 1621 AAA GAG CAA CTG AAA GCT GTT ATG GAT GAT TTC GCA GCT TTT GTA GAG AAG TGC TGC AAG 1680
 541 K E Q L K A V M D D F A A F V E K C C K 560

 1681 GCT GAC GAT AAG GAG ACC TGC TTT GCC GAG GAG GGT AAA AAA CTT GTT GCT GCA AGT CAA 1740
 561 A D D K E T C F A E E G K K L V A A S Q 580

 1741 GCT GCC TTA GGC TTA TAA CAT CTA CAT TTA AAA GCA TCT CAG 1782
 581 A A L G L * 585

Figure 15D